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VEGETATIVE PROPAGATION OF CONIFERS

XIV. NOTE ON SEASONAL PROGRESS OF ROOTING OF NORWAY SPRUCE CUTTINGS[1]

BY N. H. GRACE[2] AND J. L. FARRAR[3]

Abstract

Dormant Norway spruce cuttings were placed in outdoor propagation frames in early November; 25% of the cuttings had callus formation by the end of the following June and 80% were rooted by mid-July. Percentage rooting increased gradually to the final average value of 95% in early September. In mid-July cuttings had initiated 72% of the final number of roots though there was a gradual increase until early September. Mean root length increased by over 400% during the 20 days from July 18 to Aug. 7, the subsequent rate of increase in root length fell to less than 50% for the following 23 days. By Sept. 23 root growth had ceased; 84% of the final value had been attained by the end of August.

Introduction

Root initiation and seasonal progress of growth by conifer cuttings is of both theoretical and practical interest. Considerable information is available on rooting of Norway spruce (*Picea Abies* (L.) Karst.) cuttings in greenhouse and outdoor propagation frames (1-11). While the results of greenhouse propagation have provided limited information on time for root initiation and the order of subsequent root growth (1, 2, 6, 9-11), such detailed information is not available for outside propagation conditions. Determination of seasonal rooting progress should indicate the period over which cuttings require the greatest care and when they can be transplanted. This communication describes seasonal observations on the rooting of a late autumn planting of Norway spruce cuttings. Dormant material was used as it has been shown to root in high percentages under outside conditions (4, 5, 7, 8).

Experimental

Branches were taken from the lower third part of the tree (6) from a plantation (7) at the Petawawa Forest Experiment Station, Chalk River, Ont., in early November, 1940. Cuttings from 3 to 6 in. long were prepared from the whole length of the current season's growth and planted immediately in a medium of two volumes sand and one volume peat humus (4, 8). The outdoor

[1] *Manuscript received August 10, 1945.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, and the Dominion Forest Service, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 1283.

[2] *Biochemist.*
[3] *Forestry Engineer, Dominion Forest Service, Ottawa.*

propagation frames and care of the cuttings have been described (8). The experiment involved four randomized replicates of 15 groups each of 10 cuttings. This provided material for examination at 15 times during the season, data for each set of observations being based on four groups of 10 cuttings. The dates of removal of cuttings and the data taken are given in Table I. Cuttings were replanted after examination in order to determine the effect of removal on subsequent root initiation and growth. This procedure was discontinued as the actively growing, brittle, short roots suffered unavoidable injury.

Results

It is apparent from the data in Table I that little growth had occurred during June. However, visible growth was starting as 25% of the cuttings showed callus and 12% had new growth shoots. Twenty days later, July 18, about 80% had rooted, 5% had callus and no roots, and 40% of the cuttings had new growth shoots. By the end of August, 92% rooting had been attained. Rooting for Aug. 30 and all dates thereafter approximated 95%.

There was a progressive increase in the number of roots per rooted cutting until early in September. Data on mean root length were submitted to the analysis of variance procedure. It is apparent that slow growth occurred between Sept. 9 and 23 and that growth then ceased since substantially similar root length was obtained the following May. It is of interest to point to the better than 400% increase in mean root length for the 20 day period

TABLE I

SEASONAL PROGRESS OF ROOTING IN A NOVEMBER PLANTING OF DORMANT NORWAY SPRUCE CUTTINGS

Date of examination	Number of cuttings, %				Number of roots per rooted cutting	Mean root length, mm.		New growth shoots	
	Callused but not rooted	Rooted	With new growth shoots	With new growth shoots and rooted		Av. for each collection date	Means of averages	Number per cutting	Mean shoot length, mm.
May 21, 1941	0	0	0						
June 28, 1941	25	0	12						
July 18, 1941	5	80	40	32	2.6	10.2			
Aug. 7, 1941		75	30	22	2.8	42.4			
Aug. 30, 1941		92	55	55	3.0	60.7	61.2	2.1	16
Sept. 9, 1941		95	25	25	3.5	61.7		1.5	36
Sept. 23, 1941		97	40	40	3.6	74.6		1.2	29
Sept. 23, 1941		95	32	32	3.9	66.9	72.5	1.0	27
May 14, 1942		95			3.5	76.0			
Necessary difference, 5% level						12.2	7.9		
Sept. 23, 1941, examination of replanted cuttings of Aug. 7		70			2.9	66.0			

between July 18 and Aug. 7; over the next 23 days the increase was below 50% or about one-eighth the growth rate of the earlier period. By comparison the rate of root initiation followed an entirely different pattern. The average 2.6 roots per cutting as of July 18 represented 72% of all roots formed, taking 3.6 roots as the average final value. The inherent properties of the vegetative material could be expected to affect root initiation more strongly than the medium in which propagation occurred. Rate of root growth on the other hand was probably affected by the favourable medium.

The inherent variability of new growth development is brought out in Table I. This is probably related to the use of material from the lower part of the tree and not to the propagating medium, which has been shown to be highly favourable to occurrence and development of new growth (4, 5, 7, 8). Record of new growth was discontinued when the variability became apparent. New shoots appeared on 12% of the cuttings before any roots had been formed.

These results demonstrate that root initiation occurs rapidly and over a short period. Callus formation to the extent of 25% can be considered as the early phase of root initiation and occurred between two and three weeks after Norway spruce trees in the plantation showed new shoot growth. In early September about 85% of the total root length had been attained. Thus, it should be practicable to transplant rooted cuttings during September as appreciable growth has ceased, unless, of course, other factors, such as frost heaving of transplants, render this inadvisable. It is also concluded that cuttings require the greatest care in the propagation frame up to about mid-July. Presence of roots on a great majority of cuttings after this date can be expected to result in reduced sensitivity to moisture maintenance and shading.

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PHYSIOLOGICAL STUDIES ON SAP FLOW IN THE SUGAR MAPLE, *ACER SACCHARUM* MARSH.[1]

By L. P. V. Johnson[2]

Abstract

Sap flow from the stumps of sugar maples was over five times as great as that from the trunks of the same trees, which were detached in November, January, and March. There was no consistent variation in the sugar concentration of sap samples drawn from stumps and trunks, but samples taken from points above girdles were consistently higher in sugar than those taken from below.

Sap flow is attributed mainly to the activities of living cells, and it is suggested that the oxygen concentration of the sap and the release of hydrostatic pressure in tapped trees are related to cell activities.

The beneficial effect of alternating cold nights and warm days on sap flow is attributed to an increase in availability of oxygen to the living cell through the agency of the relative solubility levels of oxygen in water at low and high temperatures.

Introduction

Since 1873 questions related to sap flow in the sugar maple have been studied by scientific investigators (5). Yet, in reviewing the work in 1938, Miller (6) found it necessary to state that "The causes for this escape of sap . . . . are as yet little understood."

It is hoped that the present work, by virtue of its being a new approach to the problem, may add something to the accumulation of knowledge that will ultimately lead to a solution.

Materials and Methods

A group of 14 sugar maples growing on roughly the same site and being reasonably comparable as to size and crown exposure were selected for study. Each of eight of these trees was growing within about a yard of a tree suitable for providing support for its trunk (later detached and trussed perpendicularly to the companion tree). Two larger trees, Nos. 15 and 16, were selected for studies on sap and sugar production at different levels of the trunk. All trees are described in Table I.

Ages were determined from increment borer cores and crown indexes by estimation of total leaf area for each tree. Heights were measured with an hypsometer and diameters, two feet from the ground, with a diameter tape.

The general method of study, cutting and girdling at different times, sampling sap from sealed stumps and trunks, etc., will be readily apparent upon examination of Table II. Cutting and girdling were done at freezing temperatures. Trunks were cut so as to give a stump height of 2 ft.

[1] *Manuscript received June 11, 1945.*

Contribution from the Division of Applied Biology, National Research Council Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 1347.

[2] *Geneticist.*

TABLE I

GENERAL DESCRIPTION OF TREES

Tree No.	Age	Diameter, in.	Height, ft.	Crown index
1	18	4.5	33	30
2	16	4.7	35	35
3	28	4.7	37	30
4	28	4.3	30	25
5	28	4.9	32	30
6	18	4.5	38	35
7	27	4.9	35	27
8	19	4.5	37	40
9	18	4.7	37	35
10	18	3.8	35	30
11	22	4.7	32	35
12	17	3.8	35	30
13	16	3.7	33	32
14	23	3.8	37	30
15	65	11.0	72	120
16	56	9.7	70	100

Cut surfaces were sealed with marine glue and 10-oz. canvas. For sealing a stump or trunk, a 12 in. square of canvas coated with a $\frac{1}{8}$ in. layer of glue was tied, glue next to the cut surface, securely over the cut end. For sealing a girdle, a strip, wide enough to extend about two inches on either side of the cut and long enough to overlap, was similarly used. These seals were entirely efficient when applied on dull, cold days to relatively dry surfaces.

The trees were tapped by drilling holes with a sharp bit to depths of 1 in. in Nos. 1 to 14 and of 2 in. in Nos. 15 and 16. The rolled-metal tapping spout was forced through a hole in the side of the bucket as the spout was driven into the tree. This permitted the placing of tight covers on all buckets. Tapping for Expt. Nos. 1 to 12 and 17 to 26 inclusive (Table II) was done on Mar. 15, for Nos. 27 to 32 on Mar. 17, and for Nos. 13 to 16 on Mar. 31.

Trees comparable to those used in the experiments were cut, but not sealed, on Nov. 15 and Jan. 12, and similar trees were girdled but not sealed on Nov. 15 and Mar. 15. These trees provided opportunities for direct observation of sap flow at the time the experimental trees were sealed.

Sugar concentration of the sap was determined by polarization in sodium light.

Experimental Results

The results of the investigation are presented in Table II.

Considering first the sap flow from stumps and trunks (Expt. Nos. 1 to 16), it will be noted that stumps consistently yielded substantially more sap than their severed trunks, the ratios for the November, January, Mar. 15, and Mar. 30 cuts being 3.3 : 1.0, 5.4 : 1.0, 15.0 : 1.0, and 8.3 : 1.0, respectively. The average sap production (litres) from the stumps is 3.4 as compared to 0.6 for the trunks and 4.7 for the controls (Expt. Nos. 25 and 26).

TABLE II

VOLUME AND SUGAR CONCENTRATION OF SAP FROM EXPERIMENTALLY TREATED SUGAR MAPLE TREES, NOVEMBER, 1943, TO APRIL, 1944

Expt. No.	Treatment of tree	Date	Position of spout	Tree No.	Duration of sap flow, days	Volume of sap, litres	Dextrorotatory power (1 dm.)	
							1st coll.	2nd coll.
1	Cut; stump and trunk sealed	Nov. 15	Stump	1	40	3.0	3.97	4.32
2	Cut; stump and trunk sealed	Nov. 15	Trunk	1	6	0.8	—	—
3	Cut; stump and trunk sealed	Nov. 15	Stump	7	42	1.5*	3.33	3.52
4	Cut; stump and trunk sealed	Nov. 15	Trunk	7	30	1.2	—	—
5	Cut; stump and trunk sealed	Jan. 12	Stump	3	42	2.6	4.92	5.19
6	Cut; stump and trunk sealed	Jan. 12	Trunk	3	23	0.8	—	5.06
7	Cut; stump and trunk sealed	Jan. 12	Stump	4	42	2.8	—	8.87
8	Cut; stump and trunk sealed	Jan. 12	Trunk	4	23	0.2	—	—
9	Cut; stump and trunk sealed	Mar. 15	Stump	5	41	1.3	3.26	4.34
10	Cut; stump and trunk sealed	Mar. 15	Trunk	5	14	0.2	—	2.37
11	Cut; stump and trunk sealed	Mar. 15	Stump	8	42	6.2	2.58	3.57
12	Cut; stump and trunk sealed	Mar. 15	Trunk	8	8	0.3	—	—
13	Cut; stump and trunk sealed	Mar. 30	Stump	12	38	4.9	3.25	3.91
14	Cut; stump and trunk sealed	Mar. 30	Trunk	12	23	0.6	4.30	8.51
15	Cut; stump and trunk sealed	Mar. 30	Stump	6	38	2.6	3.84	4.55
16	Cut; stump and trunk sealed	Mar. 30	Trunk	6	11	0.3	—	3.98
17	Girdled and sealed	Nov. 15	Above girdle	9	42	2.6	4.74	5.26
18	Girdled and sealed	Nov. 15	Below girdle	9	42	6.5	4.09	3.88
19	Girdled and sealed	Nov. 15	Above girdle	10	30	3.5	4.56	3.16
20	Girdled and sealed	Nov. 15	Below girdle	10	30	1.6	3.79	2.29
21	Girdled and sealed	Mar. 15	Above girdle	13	31	2.8	3.96	4.02
22	Girdled and sealed	Mar. 15	Below girdle	13	31	3.3	3.44	3.38
23	Girdled and sealed	Mar. 15	Above girdle	14	41	3.0	3.82	4.45
24	Girdled and sealed	Mar. 15	Below girdle	14	42	4.6	3.68	4.01
25	Tapped normally (check)	Mar. 15	3 ft.	2	39	2.1	5.39	5.71
26	Tapped normally (check)	Mar. 15	3 ft.	11	32	7.3	3.73	3.82
27	Tapped at height of 3 ft.	Mar. 17	3 ft.	15	32	21.7	3.67	4.47
28	Tapped at height of 13 ft.	Mar. 17	13 ft.	15	31	5.0	2.15	2.87
29	Tapped at height of 23 ft.	Mar. 17	23 ft.	15	19	2.6	3.83	8.85
30	Tapped at height of 3 ft.	Mar. 17	3 ft.	16	42	64.0	3.56	3.88
31	Tapped at height of 13 ft.	Mar. 17	13 ft.	16	40	24.8	3.91	4.47
32	Tapped at height of 23 ft.	Mar. 17	23 ft.	16	32	10.9	3.91	4.77

* *Considerable leakage from stump of tree No. 7 (estimated at 2 litres).*

Observations on trees cut but not sealed showed that in November sap was exuded more readily and in greater amount from the trunk than from the stump. In January the exudation was about equal from stumps and trunks.

Sugar concentrations of sap derived from stumps and from trunks of the same tree vary inconsistently and no significant relation could be demonstrated.

The girdling experiments (Nos. 17 to 24) show consistently a higher sugar content in sap extracted from a point above the girdle than in sap extracted below the girdle. This is particularly marked for girdles made in November.

Observations on test girdles (not sealed) made in November and March showed considerable sap exudation from the exposed wood with little or none from the exposed cuts in the bark, which is in agreement with the findings of Adams (1).

The experiments on tapping at different heights (Nos. 27 to 32) show a striking reduction in sap production as the spouts were placed successively higher. The sugar concentration of the sap tends to increase as the sap is extracted from higher points in the tree.

Discussion and Conclusions

A striking feature of the results is the fact that the sap production from stumps was over five times as great as that from trunks. This is contrary to Wiegand's conclusion (8) that the source of pressure for sap flow in the maple is mainly in the trunk and branches.

The question of the relative sap content of the stump (comprised largely of the root system of the tree) and of the trunk at the time of being sealed is an important consideration in this connection. Jones and Bradlee (4) give the following average water percentages (wet weight basis) for outer wood (new wood corresponding to the wood of the young trees studied herein), top wood, and roots of the sugar maple: 31.97, 32.63, and 31.72, respectively. Extreme ranges in water percentage for the months November to April, inclusive, are: outer wood, 31.21 to 36.84; top wood, 31.04 to 35.50; and root, 30.15 to 37.51. Since sap content of the wood is closely related to the water content, it seems safe to assume that, in the present experiments, the percentage of sap in the trunk was approximately equal to that in the root—or to that in a normal tree.

Yet, upon tapping, the flow from the sealed stump approached that of the control while the flow from the detached trunk was only a small fraction as great. This implies that the force that causes sap flow remained more or less normally functional in the root and stump, but had been seriously disrupted in the severed trunk.

The results of Jones, Edson, and Morse (5), who found that positive root pressure in the sugar maple was infrequent and slight, and those of Gibbs (3) who reported the root system of an individual sugar maple to be confined to the upper two feet of soil (the ground was possibly frozen to this depth at the time of the present experiments) would appear to weaken any theory postulating a general hydrostatic pressure originating as osmotic or imbibitional forces, or both, in the epidermis, cortex, and endodermis of the root. A more plausible explanation would be that of Jones *et al.* (5), which attributes sap flow in the sugar maple to the activities of living cells, though their suggestion that temperature and gas pressure changes in the upper tree act as stimuli to these activities loses weight in the comparative absence of a trunk. Since there is little pressure created in the closed 'hydraulic' system of the tree it may be assumed that 'bleeding' from living cells does not operate against pressure, but becomes active upon a release from pressure such as is occasioned by tapping. The present results indicate that bleeding is activated below the point of pressure release, or that the capacity to bleed or to exert bleeding

pressure is localized in the root or the lower trunk or both. The reduced flow from successively higher tap holes in the trunk and the tendency for greater flow from the lower of two tap holes (as in the girdling experiment, where the holes were one above the other about 18 in. apart) lend support to this proposition.

It is not possible, even with the very short stumps used in the present experiment, to rule out Wiegand's interesting theory (8) that the outer (bark) ends of the radial pith-ray cells are stimulated by rising temperatures, causing in successive cells the membranes nearest the bark to be more permeable than those nearest the pith, and thus setting up a sap current and accompanying pressure from the pith toward the bark.

The common observation that sap flow is greater when freezing nights alternate with warm days may appear to be inconsistent with a theory that seeks to relate this flow to the activity of living cells, since continued high temperatures would presumably favour such activity. It is suggested that the oxygen concentration of the sap may be a limiting factor in vital cell activity. With alternation of cold nights and warm days the potential oxygen supply would be greater since the solubility of oxygen in water increases with lowering temperatures, resulting in greater oxygen uptake by the sap, and decreases with rising temperatures, resulting in increased liberation of oxygen to the living cells. It is significant that, at the high points in these diurnal temperature fluctuations, the maxima in available oxygen supply coincide with the maxima in vital cell activities.

The low temperatures involved in this fluctuation may have additional significance in promoting sugar synthesis from starch. Increased sugar content in the sap of the roots would lead to increased osmotic gradients and, soil conditions permitting, increased water intake by the roots. Jones and Bradlee (4) found, on a dry matter basis, 3.66% starch (over twice the sucrose content) in sugar maple roots during the sap season. A statement by Stevenson (7) seems worthy of mention in this connection: "The ideal climate for cane sugar production is one offering a long growing season followed by a short season sufficiently cold to check vegetative growth and enhance sucrose synthesis and storage."

The fact that the sap above the girdles was consistently higher in sugar content indicates obstruction by the girdle of sugar movement through the phloem. This is in accord with the theories of Curtis (2). It is perhaps significant that no such differences were observed in stumps and trunks where the xylem was also severed.

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FURTHER OBSERVATIONS ON THE MECHANISM OF GERMINATION OF THE CONIDIA OF VARIOUS SPECIES OF POWDERY MILDEW AT LOW HUMIDITY[1]

By Harold J. Brodie[2]

Abstract

Collected data from the observations of the author and others indicate that nine species of powdery mildew fungi, obtained from 21 different hosts, have been examined with regard to germination of the conidia at various humidities. Of these species, four, viz., *Erysiphe Polygoni* DC., *E. graminis* DC., *Uncinula Salicis* (DC.) Wint., and *Microsphaera Alni* (DC.) Wint., seem beyond doubt to produce conidia capable of germinating at low humidity. Five species do not tolerate low humidity, according to published information.

Conidia of *Erysiphe graminis*, detached from the conidiophore but adhering in chains, do not germinate in as high proportion as separate conidia from the same sample; also, the longer the chain, the lower the percentage of germination. In chains it is usually the end conidia that germinate, not those in intercalary position. These observations are discussed in the light of the theory previously put forward by the author to explain the germination of powdery mildew conidia at low humidity.

The apparent osmotic pressures of the cell sap of conidia of two powdery mildews were determined by plasmolysis with potassium nitrate. For *Erysiphe Polygoni*, the figure obtained was 63 atm., and for *E. graminis Hordei* Marchal, 68 atm. These figures are higher than any previously recorded in the literature seen by the author. The significance of this high osmotic pressure is discussed.

Study of the papillae that regularly appear on the end of conidia of *E. graminis* indicates that they are special structures with a special function and should not be considered as germ pores. It is suggested that the conidium wall is two-layered, at least in the region between adjacent conidia. Rupture of the outer part of the wall in a ring at the septum may serve to disjoin the conidia and form the papilla.

Introduction

That the conidia of certain species of the powdery mildew fungi (Erysiphaceae) are able to germinate in dry air was shown by Brodie and Neufeld (2) who confirmed the previous work of Yarwood (12) and suggested an explanation for the unusual nature of the conidia with respect to moisture requirements.

More recently, further verification has been published. Clayton (5), in a study of the effect of humidity upon the germination of nine different fungi, reported that the conidia of *Erysiphe Polygoni* from red clover, cabbage, and evening primrose germinated on dry slides at relative humidities ranging from zero to 100%. However, for *E. graminis Hordei*, almost no germination was observed at relative humidities of 88% or below.

In an extensive study of the biology of *Erysiphe graminis* in Manitoba, Cherewick (4) obtained good germination of conidia of the grain mildew from wheat, barley, oats, and *Agropyron* on dry slides at room humidity and also at zero relative humidity.

[1] *Manuscript received June 14, 1945.*

Contribution from the Department of Botany, The University of Manitoba, Winnipeg, Man., with financial assistance from the National Research Council of Canada.

[2] *Assistant Professor of Botany.*

Concerning *Podosphaera leucotricha* (E. & E.) Salmon, Dr. R. E. Fitzpatrick of the Dominion Laboratory of Plant Pathology at Summerland, B.C., reported to the writer (*in litt.* Jan. 23, 1942) that, in his experience, germination of the conidia takes place readily under much drier conditions than were found necessary for germination by Berwith (1), and that infection of apple leaves was observed in a greenhouse where the relative humidity was around 50%.

Recognizing that the conidia of certain species of powdery mildew apparently require higher humidity for germination than certain others, it now seems established that many species germinate and even cause infection under dry conditions.

The writer has had under observation several mildews not mentioned in the paper by Brodie and Neufeld (2), and has found that, in most of these, the conidia germinate well at low relative humidity. These will be dealt with in the present paper.

The ramifications of the research concerning the germination of mildew conidia have become numerous and will form the subjects of subsequent papers. At this point it was thought desirable to add certain confirmatory evidence to that already brought forth. The present paper reports: (1) an examination of a number of different species of powdery mildew fungi with regard to moisture requirements of the conidia; (2) the effect of the length of a chain of conidia of *Erysiphe graminis Hordei* upon the germination of the conidia in the chain; (3) a study of the osmotic pressure of the cell sap of conidia of *E. graminis Hordei* and *E. Polygoni*; and (4) a study of the structure of the papilla of conidia of *E. graminis Hordei*.

Effect of Relative Humidity on the Germination of the Conidia of Various Members of the Erysiphaceae

Since the writer first became interested in the ability of powdery mildew conidia to germinate at low humidity, he has from time to time had various species under observation. Several of these were reported upon previously and the list has now been extended.

As far as possible, the fungi were tested for germination on dry slides suspended over concentrated sulphuric acid. Some observations were made during the writer's summer vacations away from the laboratory, and for these a test was made only of germination on dry slides at the relative humidity of the air at the time of experiment. When possible the relative humidity of the atmosphere at such a time was determined roughly by the use of a sling psychrometer.

Data obtained by the writer since the paper of Brodie and Neufeld (2) was published are presented in the following table, together with some data from other sources included to add to the weight of evidence.

Nine species of powdery mildew fungi, obtained from 21 different hosts, have thus far been examined with regard to germination of the conidia at various humidities.

TABLE I

COLLECTED DATA SHOWING GERMINATION OF CONIDIA OF VARIOUS SPECIES OF POWDERY MILDEW AT DIFFERENT HUMIDITIES

Species	Host	Germination on dry slides over water, %	Germination on dry slides at atmospheric humidity, %		Germination at low humidity 0 — *R. H.* or approximately, %	Source of data	Temperature, ° C.	Date of observation
				R.H., %				
Erysiphe Cichoracearum DC.	*Helianthus annuus* L.	9	—	—	1.5	Yarwood, 1936 (12)	22	1935
Erysiphe graminis (DC.) (varieties)	*Hordeum vulgare* L.	24	—	—	2	Yarwood, 1936 (12)	22	1933
	Hordeum vulgare L.	5	—	—	0	Clayton, 1942 (5)	—	1942
	Hordeum vulgare L.	37	20	—	6	Brodie	23	July, 1941*
	Hordeum vulgare L.	21	22	—	22	Brodie	23	July, 1941*
	Hordeum vulgare L.	18	19	—	10	Brodie	23	July, 1942*
	Hordeum vulgare L.	—	12	40	—	Brodie	21	Nov., 1941*
	Hordeum vulgare L.	—	33	40	—	Brodie	21	Nov., 1941*
	Hordeum vulgare L.	—	14	50	—	Brodie	20	Feb., 1942*
	Hordeum vulgare L.	21	22	50	23	Brodie	20	Mar., 1942*
	Hordeum vulgare L.	18	29	46	8	Brodie	20	Feb., 1943*
	Hordeum vulgare L.	27	9	—	11	Cherewick, 1944 (4)	18	—
	Triticum aestivum L.	—	35	58	—	Brodie	22	Aug., 1941*
	Triticum aestivum L.	30	30	43	—	Brodie	22	Feb., 1941*
	Triticum aestivum L.	28	40	41	—	Brodie	22	Feb., 1941*
	Triticum aestivum L.	21	40	—	36	Cherewick, 1944 (4)	18	—
	Avena sativa L.	39	—	—	25	Yarwood, 1936 (12)	22	1935
	Avena sativa L.	30	16	—	17	Cherewick, 1944 (4)	18	—
	Poa pratensis L.	10	15	—	22	Brodie and Neufeld, 1942 (2)	22	1941
	Agropyron repens L. (Beauv.)	20	18	—	0	Brodie	25	July, 1942*
	Agropyron repens L. (Beauv.)	35	30	—	0	Brodie	23	July, 1941*
	Agropyron sp.	32	20	—	23	Cherewick, 1944 (4)	18	July, 1943
Erysiphe Polygoni DC.	*Trifolium pratense* L.	56	—	—	26	Yarwood, 1936 (12)	22	1930
	Trifolium pratense L.	76	—	—	47	Yarwood, 1936 (12)	22	1933
	Trifolium pratense L.	86	—	—	63	Yarwood, 1936 (12)	22	1935

* *Indicates data hitherto unpublished.*

** *Presumably this form: Hashioka's identification is Sphaerotheca fuliginea (Schlecht) Poll.*

TABLE I—*Continued*

COLLECTED DATA SHOWING GERMINATION OF CONIDIA OF VARIOUS SPECIES OF POWDERY MILDEW AT DIFFERENT HUMIDITIES—*Continued*

Species	Host	Germination on dry slides over water, %	Germination on dry slides at atmospheric humidity, %		Germination at low humidity 0 — *R. H.* or approximately, %	Source of data	Temperature, ° C.	Date of observation
				R.H., %				
Ersiphe graminis (DC.) (varieties)	*Trifolium pratense* L.	9	—	—	12	Clayton, 1942 (5)	20	1942
	Delphinium spp. (cult.)	24	30	—	34	Brodie and Neufeld, 1942 (2) av. of 3	22	1940
	Polygonum aviculare L.	7	—	—	33	Brodie and Neufeld, 1942 (2) av. of 3	22	1940
	Phaseolus vulgaris L.	25–77	25–77	—	0	Dundas, 1936 (6)	8–25	1936
	Phaseolus vulgaris L.	—	25	50	—	Brodie	—	Mar., 1943*
	Phaseolus vulgaris L.	0–21	—	—	7–30	Yarwood, 1936 (12)	22	1936
	Brassica oleracea L. (cabbage)	—	35	—	—	Clayton, 1942 (5)	—	1942
	Brassica sp. (mustard)	41	—	—	0.5	Yarwood, 1936 (12)	22	1936
	Brassica sp. (mustard)	30	5	61	0	Brodie	24	June, 1942*
	Aster sp.	—	16	65	0	Brodie	22	July, 1942*
	Oenothera sp.	33	15	—	9	Clayton, 1942 (5)	—	1942
Microsphaera Alni (DC.) Wint.	*Syringa vulgaris* L.	50	40	63	35	Brodie	21	Sept., 1941*
Podosphaera leucotricha (E. & E.) Salmon	*Pyrus Malus* L.	No germination below 100% *R.H.*				Berwith, 1936 (1)	?	1936
	Pyrus Malus L.	Infection of apple leaves at 50% *R.H.*				Fitzpatrick, 1942	?	1942*
Sphaerotheca Humuli (DC.) Burrill	*Rosa* sp.	14	—	—	0	Brodie and Neufeld, 1942 (2)	22	1940
Sphaerotheca Humuli var. *fuliginea* (*Schlecht*) Salmon	*Viola tricolor* L. (cult.)	24	—	—	0	Brodie and Neufeld, 1942 (2)	22	1940
	Cucumis sativus L.	No germination below 99%				Hashioka, 1937 (7)**	—	1937
Sphaerotheca pannosa (Wallr.) Lév. var. *rosae* Wor.	*Rosa* spp.	No germination below 95% *R.H.*				Longrée, 1939 (8)	—	1938
Uncinula Salicis (DC.) Wint.	*Populus balsamifera* L.	—	33	62	—	Brodie	23	Aug., 1943*

* *Indicates data hitherto unpublished.*

** *Presumably this form: Hashioka's identification is Sphaerotheca fuliginea (Schlecht) Poll.*

Of these species, four, namely: *Erysiphe Polygoni*, *E. graminis*, *Uncinula Salicis*, and *Microsphaera Alni*, seem beyond doubt to tolerate low humidity in the matter of conidium germination. Collections of conidia of such species as *E. Polygoni* from certain host plants seem better able to germinate at low humidity than conidia of the same species from other host plants.

Five species appear unable to tolerate low humidity, according to available information. It may well be, however, that more extensive study of these species will reveal a degree of tolerance beyond that indicated at present. *Erysiphe Cichoracearum*, for example, commonly develops on wild species of *Helianthus* in hot dry weather. The writer, in 1935, observed heavy development of mildew on *H. tuberosus* L. at Macdonald College, Que., during a prolonged spell of hot dry weather in August. The plants were growing in an entirely unshaded location.

The Effect of Length of Chain of Conidia upon Germination of Conidia in the Chain

From time to time it has been observed that in a large sample of spores of *Erysiphe graminis* collected on a glass slide, the spores remaining attached in chains do not germinate nearly as well as spores that have become detached. In order to obtain data that would be more reliable than casual observation, a special study was made of the germination of spores that remain attached in chains.

Erysiphe graminis growing on Renown wheat in the greenhouse was used as material, because an abundant supply happened to be available. Some studies were made in February, 1941, when the relative humidity of the laboratory air was seldom over 35%. Later observations were made in February of 1942 and 1943 when the relative humidity in the laboratory was usually about 40% as determined by the use of a sling psychrometer.

In all these experiments, no difficulty was experienced in obtaining excellent germination on dry slides exposed to diffuse light on the laboratory table. It was found that maximum germination was obtained when spores were taken from plants at about 11 a.m. All germination tests were therefore made at that hour, using freshly dislodged spores.

I. Heavily infected wheat was shaken over slides and the spores allowed to remain undisturbed for two hours. On each slide 300 single spores were counted for each figure given, as well as 300 spores adhering in chains, and the germination percentages were recorded. The results are presented in Table II.

It will be seen that, in every test, the germination percentage for conidia that adhered in chains was far lower than for single conidia.

II. An attempt was made to determine whether or not the length of a chain of conidia had any effect upon the germinability of spores in the chain when the chain was unbroken. Again 300 spores were counted for each figure given. Table III presents the results of these observations.

TABLE II

COMPARISON OF GERMINATION OF SEPARATE SPORES WITH THAT OF SPORES IN CHAINS IN THE SAME SAMPLE

Temperature, °C.	Relative humidity	Germination (%)	
		Single spores	Spores in chains
23.5	30%	21.5	7.0
22.0	20%	40.2	28.0
22.0	35%	42.0	13.8
22.0	35%	40.5	7.6
23.0	26%	28.0	12.7

TABLE III

EFFECT OF LENGTH OF CHAIN UPON GERMINATION OF CONIDIA IN THE CHAIN

Temperature, °C.	Relative humidity	Number of spores in chain						
		2	3	4	5	6	7	8
		Percentage germination						
22	35%	40.0	30.0	12.0	10.5	3.0	2.3	0
22	35%	21.0	16.1	7.0	5.0	3.8	0.0	0
23	26%	22.0	12.0	4.3	—	—	—	—

From these data it would appear that the longer the intact chain of spores, the less likelihood there is of the spores germinating. It should be noted that, except when spores were obtained from plants that had been left undisturbed for several days, long chains were rare. Chains of two, three, or four spores were the most abundant.

III. Observations indicated that when spores adhere in chains, it is usually at the end of the chain that germination takes place. The following counts were recorded.

TABLE IV

COMPARISON OF GERMINATION OF END AND INTERCALARY CONIDIA

	Number of chains examined (3 to 6 spores per chain)	Number of chains where germination began at one end	Number of chains where germination began at both ends	Number of chains where no germination took place
	15	13	0	2
	7	2	3	2
	7	3	2	2
	5	2	2	1
	3	2	1	0
	51	25	6	20
	43	20	11	12
Total	131	67 (51%)	25 (19%)	39 (30%)

In the greatest number of examples, in chains of three or more spores, germination begins at one end. In a smaller proportion it begins at both ends. No example was found of germination beginning in the middle of the chain.

Discussion of Data

The data presented in the subsections I, II, and III above, seem capable of explanation on the basis of the ideas already put forth by the writer. If the stimulus to germination of the powdery mildew conidia is the passage of oxygen into, and the passage of carbon dioxide out from, the conidium, principally by way of the papilla, then one might expect that conidia adhering in chains would not germinate in as high proportion as those that are separate. The data given in Table II appear to indicate that this is true. The intercalary conidia have access to the air only, or principally, via the end conidia, and it may well be that diffusion of gases through the end conidia to intercalary conidia—particularly those in the middle of the chain—is so slow that many do not germinate at all, evidence in support of which is brought out in Table IV. It would also follow that the longer the chain the lower would be the germination percentage, as is shown in Table III.

The Osmotic Pressure of the Cell Sap of Conidia

Brodie and Neufeld (2) observed that the conidia of *Erysiphe Polygoni* could be plasmolysed only with strong solutions of electrolytes and surmised that this indicated a very high osmotic pressure. No detailed report on the matter was given in their paper.

The writer has since re-examined the plasmolysis of the conidia of *E. Polygoni* and has attempted to determine the apparent osmotic pressure* of the cell sap of conidia of that species and of *E. graminis Hordei.*

TABLE V

PLASMOLYSIS OF POWDERY MILDEW CONIDIA BY POTASSIUM NITRATE SOLUTION

Concentration of solution required to produce plasmolysis	
E. Polygoni	*E. graminis Hordei*
1.32 *M*	1.77 *M*
1.67 *M*	1.87 *M*
1.92 *M*	1.87 *M*
1.67 *M*	1.55 *M*
1.72 *M*	1.92 *M*
1.87 *M*	2.00 *M*
1.92 *M*	1.92 *M*
1.50 *M*	1.67 *M*
Average 1.69 *M*	1.82 *M*

** In view of the peculiar nature of the conidial protoplast (2), it would appear advisable to use the qualified expression 'apparent osmotic pressure.'*

No plasmolysis of either species occurred in sucrose solutions. The conidia shrank considerably in strong sucrose solution, but no plasmolysis could be observed, probably because of the shrinkage of the entire spores.

As plasmolyte, solutions of potassium nitrate varying in concentration from 1 *M* to 2.5 *M* were used. For final determinations, graded solutions were made up differing from one another by 0.05 *M*. Conidia on slides were irrigated with different concentrations of plasmolyte until a point was found at which plasmolysis was observed in some spores. Not all conidia in any sample were plasmolysed at this point, and it was always necessary to use stronger solutions to obtain plasmolysis of the majority of spores in any field.

The results for a number of trials for two species are given in Table V.

When the average figure for each species is used as a basis of calculation, the apparent osmotic pressures of the cell sap of conidia are:

For *Erysiphe Polygoni*, of the order of 63 atm.
For *Erysiphe graminis Hordei*, of the order of 68 atm.*

Thatcher (9, 10), in reviewing the literature concerning osmotic pressure in fungi, reports several high figures. For *Pythium de Baryanum* Hesse, an osmotic pressure of 54 atm. has been recorded. The germ tubes of rust fungi also appear to have a very high osmotic pressure: Thatcher recorded 44.25 atm. for the germ tubes of *Uromyces Fabae* (Pers.) de Bary. Hyphae of *Erysiphe Polygoni* were determined by Thatcher to have an osmotic pressure of 18 atm.

From Thatcher's work it would appear that many parasitic fungi have a high osmotic pressure in the mycelium or the haustoria.

The osmotic pressure determined by the writer for the cell sap of conidia of *Erysiphe Polygoni* and *E. graminis Hordei* are higher apparently than any that have been recorded.

The significance of the high osmotic pressure is not as yet clear. As the writer has stated in a previous communication (2), the protoplast of the conidium in the Erysiphaceae probably lacks much free water. What cell sap there is may be quite concentrated. The high osmotic pressure might be an aid to the spores in absorbing water from relatively dry atmospheres, and as such is of interest. If, however, the protoplast itself is dense and if it contains, as it may well, colloidal materials of a hydrophylic nature, the absorption of water by the germinating spores (when water is present) may be partly a question of imbibition. It is probable that neither high osmotic pressure nor imbibition pressure can be of much importance when mildew

** In this determination, the temperature was 22° C. A correction was made in calculation allowing for the dissociation of potassium nitrate at 22° C. The results are, however, advisedly expressed only in two significant figures.*

conidia germinate in absolutely dry air, however, and the writer prefers not to theorize at this point until further knowledge of the nature of the protoplast is available.

In this connection, attention has already been drawn (2) to the fact that the conidium, considered separately from its germ tube, apparently does not change in volume during germination. Observations to verify this point have again been made, and in no instance has it been possible to detect any change in width or length of the conidium while it is germinating. When death ensues, a marked shrinkage takes place.

Since the production of a germ tube means an increase in the volume of the germling as a whole, when compared with the ungerminated spore, one may ask how such an increase is possible in a dry atmosphere from which the spore cannot absorb water.

Closer examination, however, reveals that the added volume is actually very small, as is shown in the following table based on actual measurements of conidia germinating on dry slides over concentrated sulphuric acid on the laboratory bench.

TABLE VI

RELATION OF VOLUME OF GERM TUBES TO VOLUME OF UNGERMINATED CONIDIA

Species	*Erysiphe graminis*	*Erysiphe graminis*	*Erysiphe Polygoni*
Length of conidium (l)	23 μ	34 μ	36 μ
Width of conidium (w)	16 μ	15 μ	18 μ
Volume of conidium $4/3\ \pi\ (\frac{1}{2}\ l)\ (\frac{1}{2}\ w)^2$	3078.8 μ^3	3981.5 μ^3	6100 μ^3
Length of germ tube (a)	7 μ	11 μ	16 μ
Width of germ tube (b)	3.5 μ	3.5 μ	4 μ
Volume of germ tube $\pi\ (\frac{1}{2}\ b)^2\ a$	67.3 μ^3	105.7 μ^3	201 μ^3
Relation of volume of germ tube to volume of ungerminated conidium	2.2%	2.6%	3.3%

Calculations made from data obtained over a period of several years indicate that *when conidia are germinating in dry air*, the short germ tubes that are developed often have a volume as small as 1.5 to 2% of the volume of the ungerminated conidia and probably seldom greater than 4%.

Evidence presented in an earlier paper by the writer (2), suggests that the protoplast of the ungerminated conidium contains very little free water. When germination begins, free water may be produced: (i) by respiration; (ii) by changes in colloidal materials containing bound water. It seems entirely within the limits of possibility that a change of volume of 2 to 4% could be accounted for by either or both of these factors.

The Structure of the Papilla of the Conidium of *Erysiphe graminis*

In *Erysiphe Polygoni*, the writer has shown (2, 3), that the conidium is formed by the development of a ring of wall material that extends toward the centre of the conidiophore until a perforate disk is formed. Later, in this species, the pore is closed leaving the conidium attached to the parent conidiophore only by a minute elevated structure to which the term papilla has been applied.

In *E. graminis*, protoplasmic strands connect all living mycelial cells (3). In chains of conidia, protoplasmic continuity is maintained even into the terminal conidium. Just when the terminal mature conidium has its protoplasmic connection with the subterminal immature conidium broken is unknown, but no evidence was found to show that the pore of the terminal conidium is actually plugged in *E. graminis* as it is in *E. Polygoni*.

From time to time, the papilla of the conidium of *E. graminis* has been studied in the living condition, and after staining, and these observations are reported herewith.

1. Fig. 5 shows the terminal conidium of a chain. The material has been stained according to the method previously described by the writer (3). The terminal conidium is apparently immature judging from its firm and broad attachment to the subterminal spore. At this stage protoplasmic continuity of the terminal with the subterminal conidium can always be demonstrated except where severe plasmolysis has taken place.

2. Fig. 7 shows a terminal conidium as it was observed with oil immersion after staining. The protoplasts of the two contiguous conidia have apparently become completely separated from one another as a result of plasmolysis. The conidia, however, are still held together by a common outer wall (Fig 7, *a*). In this particular specimen no pore between adjacent conidia could be seen, possibly because of the extreme swelling of the transverse wall, but a deeply stained area (Fig. 7 *b*) was strongly suggestive of a gap or region of separation of the two conidia. While it is recognized that the gap might have been an artifact, it is illustrated as evidence that would seem to support the ideas advanced below.

3. In Fig. 2 is represented the end of a chain of conidia (observed in living condition) from which a conidium had just been dislodged by shaking. A rim of the wall previously common to the terminal and subterminal conidia is shown. Specimens showing this rim are not commonly found. Frequently, however, scraps of the rim can be seen.

4. Looking down on the end of a conidium at different angles with the high power (dry) one cannot discern much detail. Figs. 3 and 4 illustrate what can usually be seen. The papilla appears as a raised disk or washer-like structure, the edges of which are sometimes sharply defined (as in Fig. 3) or gradually sloping (as in Fig. 4).

5. When conidia are stained following the technique developed by the writer (3), and are treated by gently warming the preparation, so as to cause the wall to swell, it is sometimes possible to find a thread of protoplasm passing part way into the wall below the papilla, as shown in Fig. 6. Dozens of stained spores were examined without a single one being found showing the protoplasm actually passing completely through the wall as it does between adjacent immature conidia. When the wall is greatly swollen, the papilla loses its definite outlines and presents the appearance of a hump much lighter in colour than the rest of the conidium wall, suggesting that the chemical nature of the papilla is not identical with that of the rest of the conidium wall.

These observations, together with those made previously on *Erysiphe Polygoni*, form the basis for the following discussion of the mode of formation, structure, and function of the papillae developed on conidia of Erysiphaceous fungi.

In both *E. graminis* and *E. Polygoni*, the conidia are formed by the development of a ring of cell wall material that is added to inwardly until a transverse disk-like septum is formed, a pore being left in the centre through which the cytoplasm of the young spore is connected with that of the conidiophore.

In *E. Polygoni* one conidium is matured and abstricted each day; whereas in *E. graminis* the conidia develop in chains, several mature conidia remaining attached to the conidiophore.

The protoplasmic connection of the freshly matured conidium is probably broken, in *E. Polygoni*, by the deposition of material of unknown nature in the septal pore. In *E. graminis*, however, all living conidia that are still firmly attached to one another in a chain are connected by protoplasmic strands; but conidia that are sufficiently mature to become dislodged seem to have had their pores plugged in some way, for it has not been possible to trace the protoplasmic strand through the wall of disjoined conidia that have been fixed and stained.

EXPLANATION OF FIGURES

Erysiphe graminis DC. Figs. 2, 3, and 4 from living unstained material. Figs. 5, 6, and 7 from fixed and stained material. [*See paper by Brodie (3)*].

FIG. 1. *Diagrammatic representation of two adjacent conidia in chain: a, conidium wall; b, c, outer layer of wall; d, inner layer of wall extending to form transverse septum; e, pore.* × *4000 approx.*

FIG. 2. *End of conidium showing rim occasionally seen.* × *2500.*

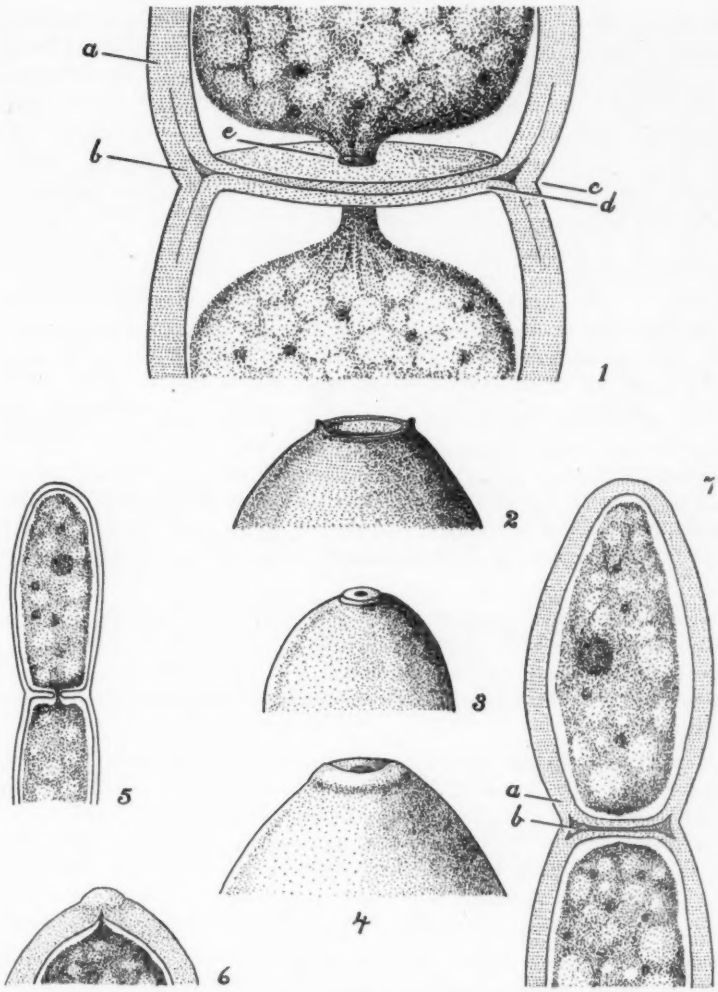
FIG. 3. *End of conidium showing washer-like papilla.* × *1600.*

FIG. 4. *End of conidium showing usual crater-like appearance of papilla.* × *2500.*

FIG. 5. *Apex of chain of stained conidia showing protoplasmic connection between adjacent protoplasts.* × *900.*

FIG. 6. *Papilla end of stained conidium: protoplasmic thread attached to wall but not passing into papilla; papilla lighter in colour than rest of wall.* × *1800.*

FIG. 7. *Apex of chain of stained conidia: a, outer wall swollen by warming stained preparation; b, gap between conidia indicating (?) disjunction of conidia; protoplasmic connection between conidia broken by plasmolysis.* × *1800.*



The nature of the plug is unknown, but evidence has already been presented (2) that shows that although an actual hole is not left in the wall, the centre of the papilla is a region of permeability much higher than that possessed by the rest of the conidium wall.

Just how the conidium is actually disjoined is difficult to determine. When the break occurs, one commonly finds the conidiophore or (in chain formers) the next conidium, showing scraps of wall material adhering in a ring (Fig. 2). This suggests that the conidial wall at maturity is double, as is indicated by

the diagram shown as Fig. 1. If the outer part of such a double wall were to break in a ring (beginning at *c* in Fig. 1), then the observation shown in Fig. 2 is explained. Evidence for a double structure of the cell wall in fungi has been given by Wahrlich (13).

In all species examined by the writer, disjunction of the conidia is passive, a shock or a puff of wind being necessary to free mature conidia. After the conidium has been freed, some changes must take place in the papilla. The turgidity of the spore would probably distend the thin part of the transverse septum (Fig. 1, *d*), causing it to be raised above the level of the spore wall, as it actually appears (Figs. 3 and 4). The remnants of the outer half of the wall (Fig. 1, *b*, *c*) might act as a restricting band giving the sharp margin to the papilla frequently observed (Fig. 3). In other spores the amount of this outer wall remaining would be slight and the papilla would present the appearance of a smooth hump, as it commonly does (Fig. 4).

Pores to facilitate the emergence of germ tubes through the thick wall of spores of many kinds of fungi are well known, those of the urediniospores and teliospores of the rust fungi being examples. In the Erysiphaceae, however, as far as the writer has been able to determine from the literature or personal observation, the *germ tube does not emerge through the papilla*. That structure would therefore appear to have a different function from the germ pores of other fungi.

It has already been suggested that the papilla constitutes a permeable spot in an otherwise relatively impermeable cell wall and that its significance lies in the fact that it allows the ready passage of gases, the release of carbon dioxide from the conidium and the entrance of oxygen being prerequisite to germination (2).

As long as a conidium remains attached, the permeable area is still sealed in by the outer layer of the wall (Fig. 1, *b*, *c*) and the spore remains in a dormant or static condition. Upon being abstricted, the permeable papilla area is exposed and gaseous exchange becomes possible, leading to germination. This theory requires that the spores should not germinate *in situ*, and the writer has insisted that, under natural conditions, he has never observed spores germinating *in situ*, i.e. while still attached to the parent conidiophore.

Recently Cherewick (4) asserted that germination *in situ* occasionally takes place, and published photographs illustrating its occurrence in *E. graminis*. It should be noted, however, that Cherewick observed this only under conditions that would obtain only occasionally during the life of the fungus. The writer believes firmly that Cherewick's observation can be attributed to rather unusual conditions. In extensive observations of various species of powdery mildew over a period of eight years, the writer has never once found a germ tube on any conidium that was still attached to the parent conidiophore. It is possible that in a species such as *E. graminis* some conidia in a chain might germinate if the attachment between two adjacent conidia had been ruptured.

This raises a point of some theoretical importance. One might ask, why is it that in chain-producing species of powdery mildew, after the terminal conidium has been abstricted, the conidia that remain attached to the parent conidiophore do not germinate? The conidium, the papilla of which lies adjacent to the one that has been freed, would be exposed to gaseous exchange and might be expected to germinate like the one just freed. This apparently does not occur, and the writer can only suggest, at this stage of the investigation, that some changes take place in the papilla of the remaining terminal conidium that do not take place in the papilla of the freed conidium—changes that may make the wall at that point impermeable. Investigation into this problem is already in progress.

Acknowledgment

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THE EFFECT OF LEAF RUST OF BARLEY ON THE YIELD AND QUALITY OF BARLEY VARIETIES[1]

By Margaret Newton[2], B. Peturson[3], and W. O. S. Meredith[4]

Abstract

An experiment was carried out to test the effect of leaf rust of barley (*Puccinia anomala* Rostr.) on the yield, grade, and malting quality of the six barley varieties, O.A.C. 21, Mensury, Chevron, Peatland, Regal, and Plush. Leaf rust reduced the grade of O.A.C. 21 and Mensury by one commercial grade, and caused statistically significant reductions in the yield, bushel weight, and kernel weight of all the varieties tested except Mensury. It adversely affected the value of all the varieties for malting purposes by reducing the percentage of heavy-grade kernels. The nitrogen content and the wort nitrogen content were reduced by leaf rust, but the malt extract and diastatic powers were not greatly affected. Differential responses of the varieties to rust infection were observed in yield, kernel weight, bushel weight, and malt properties.

Introduction

Leaf rust of barley (*Puccinia anomala* Rostr.) occurs to some extent in all the nine provinces of Canada. Although usually much less prevalent than stem rust it sometimes causes heavy infections, generally in quite localized areas. This rust thrives best under comparatively cool conditions and consequently is more prevalent during cool summers, and is usually most abundant in the cooler areas of Canada, such as the Maritime Provinces and British Columbia, although severe local attacks have been reported from the other provinces. As little or no information was available regarding the extent to which leaf rust may damage a crop, an investigation was undertaken at Winnipeg to determine the effect of this rust on the yield and quality of six barley varieties, four of which are widely grown in Canada. Data for only one year (1941) are available as, owing to war conditions, the investigation had to be discontinued after the first year.

Materials and Methods

For the tests, two types of six-rowed barley were used—O.A.C. 21 and Mensury, varieties of high malting quality, and Chevron, Peatland, Regal, and Plush, varieties inferior in malting quality to O.A.C. 21 and Mensury. The varieties were grown in rod-row plots. Each plot consisted of three rod-rows spaced one foot apart. There were 12 plots of each variety. The

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plots were arranged in randomized blocks each of which was split into two equal sub-blocks consisting of one plot of each variety, that is, each variety appeared once in each sub-block. The plants of one sub-block in each block were inoculated with leaf rust when the plants had reached the 5-leaf stage. The plants in the uninoculated sub-blocks were kept as free from rust as possible by dusting them three times a week and after every heavy rain with sulphur (Kolodust) at the rate of 30 lb. per acre per dusting. Dusting was continued until one week before harvest. Centre rows only of each plot were harvested.

Leaf rust percentages were estimated while the leaves were still green. The estimate of rust percentages was based on the scale of percentages adopted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. For each individual plot, the yield, 1000-kernel weight, and bushel weight were taken and composite samples of each variety from the check and from the rusted plots were tested for malting quality by the standard procedures at the Malting Laboratory of The University of Manitoba (4).

Experimental Results

Field Tests

As shown in Table I, all the artificially-inoculated plants became heavily rusted. Only very slight traces of leaf rust developed in any of the dusted plots, and stem rust and other diseases were almost wholly absent from all plots.

The results presented in Table I show that leaf rust of barley had an adverse effect on the yield, kernel weight, and bushel weight of all the six varieties tested. The two high-quality malting varieties, O.A.C. 21 and Mensury, were injured less by leaf rust than were the other four varieties, This was particularly noticeable with regard to the variety Mensury, which. although almost as heavily rusted as some of the other varieties, suffered proportionately much the least rust injury. The results of the statistical analysis of the yield, kernel weight, and bushel weight data are given in Table II. As indicated in this table, the mean squares for these three factors due to the effect of rust infection were all highly significant. This table also shows that, for kernel weight and bushel weight, the mean squares due to the interaction, rust $\times$ varieties, were significant, indicating a differential response of the varieties to rust infection with regard to these factors. The mean square for yield due to the interaction, rust $\times$ varieties, although not significant, approached the level of significance. The mean squares due to differences between varieties were all highly significant.

Although leaf rust affected the bushel weights of O.A.C. 21 and Mensury much less adversely than it affected the bushel weights of Chevron, Peatland, Regal, and Plush, it reduced the grade of O.A.C. 21 and Mensury by one commercial grade (Table I) but not the grade of the other four varieties.

TABLE I

COMPARISON OF YIELD, KERNEL WEIGHT, BUSHEL WEIGHT, AND GRADE OF GRAIN SAMPLES OF BARLEY VARIETIES FROM CHECK AND RUSTED PLOTS

Variety	Treatment	Severity of infection	Average yield per acre, bu.	Reduction in yield, %	Average weight per 1000 kernels, gm.	Reduction in kernel weight, %	Average weight per bushel, lb.	Grade
O.A.C. 21	Check	Tr.	76.25		39.84		50.3	1 C.W.
O.A.C. 21	Rusted	58.3%	65.90	13.57	36.18	9.19	48.8	2 C.W.
Mensury	Check	Tr.	63.83		38.54		50.5	1 C.W.
Mensury	Rusted	55.0%	62.80	1.62	36.52	5.25	49.9	2 C.W.
Chevron	Check	Tr.	59.19		34.46		49.6	3 C.W.
Chevron	Rusted	67.5%	45.56	23.03	29.04	15.73	46.0	3 C.W.
Peatland	Check	Tr.	67.08		34.42		50.5	3 C.W.
Peatland	Rusted	79.1%	56.30	16.08	28.70	16.62	47.5	3 C.W.
Regal	Check	Tr.	73.53		39.26		50.5	3 C.W.
Regal	Rusted	87.5%	56.35	23.04	32.90	16.20	47.5	3 C.W.
Plush	Check	Tr.	86.35		44.68		49.5	3 C.W.
Plush	Rusted	63.3%	66.81	22.63	36.92	17.37	46.5	3 C.W.
Necessary difference*			9.70		2.38		1.37	

* *Necessary difference, 5% level of significance, between means of rusted and check plots within a given variety.*

TABLE II

ANALYSES OF VARIANCE OF YIELD, BUSHEL WEIGHT, AND KERNEL WEIGHT DATA

Source of variance	Degrees of freedom	Mean squares		
		Yield	Bushel weight	Kernel weight
Blocks	5	74.156	6.254*	11.830*
Varieties	5	827.192**	10.004**	157.132**
Error (*a*)	25	33.730	1.215	2.747
Rust	1	2614.850**	124.293**	508.273**
Rust × varieties	5	131.307	4.484*	11.277*
Error (*b*)	30	69.213	1.407	4.191

* *Significant at the 5% point.*
** *Significant at the 1% point.*

This seeming anomaly was due entirely to regulations governing the grading of these varieties. O.A.C. 21 and Mensury are eligible for Grade 1 C.W. Six-row, a grade requiring a sample weight of 50 lb. per measured bushel, and consequently a slight reduction in weight per bushel may result in loss of grade. The four varieties Chevron, Peatland, Regal, and Plush, owing to

their low malting quality, are excluded from grades higher than 3 C.W., a grade of relatively low bushel weight (46 lb.) and, therefore, unless their reduction in bushel weight is pronounced, their grade is not affected.

Discussion

As shown above, the reduction in yield, kernel weight, and bushel weight is not always directly proportional to the amount of rust infection present on a variety. This fact was particularly noticeable in the varieties Mensury and Plush. Although the infection on Mensury averaged about the same as that on Plush, the yield, kernel weight, and bushel weight of Mensury were only slightly affected, whereas those of Plush were markedly reduced. In this experiment, the differential effect of rust on these varieties cannot be attributed to the differences in earliness of the varieties tested, for Mensury, one of the earliest maturing varieties in the test, ripened only two days earlier than Plush, the latest maturing variety tested. Furthermore, the rust infection had reached maximum intensity on all the varieties about two weeks before they matured. This differential response of varieties to heavy rust infection was observed by Caldwell *et al.* (2), who found that, although in most varieties of wheat the loss due to leaf rust was approximately proportional to the severity of infection, in one variety, Fulhard, the yield was not appreciably reduced by heavy rust infection. At present it is not definitely known why some cereal varieties apparently can tolerate rust infection better than others.

The data presented indicate clearly that even a moderately heavy infection of leaf rust may cause very appreciable damage to barley. For this reason, in any area where weather conditions are likely to favour leaf rust development, the growing of varieties susceptible to this rust definitely increases the hazard to barley production.

Malting Tests

Effect of Leaf Rust on Malting Quality

Limitations of space render it impossible to discuss in detail the definition of terms and the interpretation of data representing malting quality. These have been discussed fully elsewhere (1) and are briefly summarized as follows: Malt extract represents the amount of soluble material derived from malt under specific mashing conditions, and it is a quantitative measure of amount of material that can be converted into alcohol or other products. Diastatic power is a measure of the ability of the malt to convert the insoluble starch into soluble sugars and it is particularly important when malt is used to convert raw grains into soluble material. Wort nitrogen is the amount of nitrogen that appears in the malt extract, expressed as percentage of the wort (or extract) solids, and is a useful index of the yeast feeding properties of the wort. Sprouts are the amount of rootlets produced during the malting process, and malt yield is the percentage of original barley recovered as malt.

The mean values of each of the more important malting properties for each check sample of each variety are given in Table III, together with the differ-

TABLE III

COMPARISON OF MALTING QUALITY OF GRAIN SAMPLES FROM CHECK AND RUSTED PLOTS

Variety		Barley		Malt			Malting	
		Heavy kernels, %	Nitrogen, %	Extract, %	Diastatic, power, °L.	Wort nitrogen, %	Sprouts, %	Malt yield, %
O.A.C. 21	1	93.2	2.50	74.4	145	1.34	7.0	86.4
	2	−10.2	−0.10	−0.4	−1	−0.06	−0.2	0.7
Mensury	1	93.7	2.54	75.0	150	1.36	6.7	86.3
	2	−5.9	−0.08	−0.6	4	−0.04	0.1	0.4
Chevron	1	77.5	3.18	70.8	211	1.60	6.4	87.8
	2	−39.8	−0.20	−0.2	−12	−0.06	0.6	−1.0
Peatland	1	86.0	2.92	72.1	156	1.40	7.0	87.4
	2	−35.8	−0.24	−0.3	−14	−0.08	0.2	0.2
Regal	1	92.7	2.64	71.6	86	1.28	5.6	87.4
	2	−21.5	−0.33	0.8	−4	−0.20	0.5	−0.4
Plush	1	94.8	2.66	72.1	118	1.10	5.4	88.2
	2	−9.6	−0.27	0.1	−18	−0.07	0.3	0.1
Mean	1	89.6	2.74	72.6	145	1.34	6.3	87.2
	2	−20.4	−0.20	0	−8	−0.08	0.3	0
Necessary difference between pairs		1.5	0.05	0.6	12	0.07	0.7	1.4
Necessary difference between varietal means		26.2	0.17	0.9	15	0.10	0.5	1.0

Note: 1 = Check sample.
2 = Difference between check and rusted samples. Check samples used as base.

ences between these and the mean values for the rusted samples. The mean values over all varieties, are also given. The data for each determination were subjected to separate analyses of variance. The results of these are given in Table IV and the necessary differences in Table III were calculated from the appropriate mean squares. From these it is evident that rust infection, on the average, had significant effects on percentage heavy kernels, barley nitrogen content, and wort nitrogen content. In each of the first two properties the mean square due to interaction between varieties and rust infection was significant. The interaction mean squares for extract, diastatic power, and wort nitrogen approach a significant level. In other words, the varieties did not react to rust infection to the same degree, particularly in the barley properties.

The mean values, over all varieties, in Table III show that rust infection caused decreases in percentage heavy kernels, and barley nitrogen content. Comparisons between individual varieties show that the differences in degree

TABLE IV

ANALYSES OF VARIANCE OF MALTING DATA

Determination	Mean squares			
	Varieties	Rust	V × R	Duplicates
Heavy kernels, %	740.86	2509.21*	208.44**	0.46
Nitrogen, %	0.2493**	0.2380**	0.0092**	0.0005
Extract, %	9.2977**	0.0066	0.2467	0.0908
Diastatic power, ° L.	6844.2**	337.5	72.3	31.7
Wort nitrogen, %	0.1190**	0.0409*	0.0030	0.0011
Sprouts, %	1.5990**	0.4267	0.0727	0.1100
Malt yield, %	1.5674*	0.0004	0.3714	0.4438
Degrees of freedom	5	1	5	12

* and ** *Significantly greater than greatest mean square affecting differentiation. Double asterisk denotes that the 1% level of significance was attained. Single asterisk denotes that the 5% level of significance was attained.*

of damage due to rust are striking. Mensury was affected the least in barley qualities, followed closely by O.A.C. 21, while the changes in the other varieties were considerably greater.

The effects of rust on properties measured in the malt are not as great as those measured in the barley, but varietal differences in reaction are also evident. The malt extract of Mensury was reduced significantly by rust, while that of Regal was increased. O.A.C. 21, Mensury, and Regal show distinct differences from the other varieties with respect to change in diastatic power, and O.A.C. 21, Mensury, and Chevron differ from the other varieties with respect to change in wort nitrogen content.

Discussion

The effect of rust on the barley properties is not unexpected in view of the reduction in yield. At first sight, the reduction in nitrogen content appears somewhat anomalous, as nitrogen content frequently increases with decrease in kernel weight (4, 6, 7). This reduction in nitrogen content is not unexpected, however, as similar results have been obtained with wheat (2, 3, 5, 8). When the nitrogen content of the different varieties is calculated as grams per 1000 kernels, as shown below, additional information is obtained.

GRAMS NITROGEN PER 1000 KERNELS[1]

——	O.A.C. 21	Mensury	Chevron	Peatland	Regal	Plush
Check	0.89	0.90	1.06	0.96	0.93	1.05
Rusted	0.81	0.86	0.85	0.79	0.78	0.85
Difference	0.08	0.04	0.21	0.17	0.15	0.20

[1] *The kernel weights used in the calculations were obtained from the composite samples prepared for malting and the results are expressed on dry basis.*

The reduction in yield and kernel weight caused by rust shows that carbohydrate deposition was retarded and the reduced amount of nitrogen per 1000 kernels in the rusted samples show that protein deposition was also retarded. Further, the rust infection did not affect protein and carbohydrate deposition to the same extent. The indications from this experiment are that rust infection interfered with plant metabolism at an early stage.

The differential responses of the varieties to rust that were noted in the yield data are also evident when barley properties are considered. Mensury, which was scarcely affected in yield by rust, was reduced in malting quality only by a small reduction in percentage heavy kernels. O.A.C. 21 was reduced materially in yield, but the change in malting quality was relatively small and also confined to barley properties. The main effect of rust was to reduce percentage of heavy kernels, and kernel weight. The other varieties, Chevron, Peatland, Regal, and Plush, were reduced considerably in yield and in malting quality. The decrease in malting quality was also mainly confined to percentage heavy kernels and kernel weight. The results suggest that Mensury and, to some extent, O.A.C. 21 possess a much higher tolerance to leaf rust than other varieties. Both varieties were infected quite heavily with leaf rust, but the effects in yield and quality were small.

The effects of rust in the malt properties—extract, diastatic power, and wort nitrogen—are mainly in accord with expectations based on the differences between paired samples in nitrogen content (4). Decreases in barley nitrogen content are usually accompanied by increase in malt extract, but in this set of samples the reduction in kernel weight should cause a decrease in malt extract. On the whole, then, the amounts of malt extract obtained from the samples from the rusted plots are somewhat higher than would be expected. The gain in extract is particularly pronounced for Regal and this variety also shows a decrease in malt diastatic power that is considerably less than would be expected on the basis of the differences in nitrogen content. This variety is known to be deficient in enzymes and thus it does not modify well during malting. As the sample from the rusted plots of this variety, and also those of other varieties, modified fairly well in spite of the effect of rust on the barley properties, it seems logical to suggest that the carbohydrate materials of the barley were affected by rust in some way and made more readily available as malt extract. As noted, the data in this test are few, but the effect of rust on physiological activity seems worthy of further attention.

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SEDIMENTATION AND DIFFUSION MEASUREMENTS ON GLUTENS FROM WHEATS OF WIDELY DIFFERING QUALITY[1]

By A. G. McCalla[2] and Nils Gralén[3]

Abstract

Gluten was washed from three flours of widely varying quality. Sedimentation studies showed that the proportion of the gluten that was molecularly dispersed varied from 34 to 68% of the total. The amount so dispersed was reduced by an increase in amount of fatty acids present in or added to the flour, and by a decrease in the amount of ether-insoluble lipoids. No other basic differences in the fundamental properties of the dispersed protein, as determined by sedimentation and diffusion methods, could be related to the differences in gluten quality.

Introduction

Many different methods have been used in attempts to obtain quantitative measurements of the variability in the properties of gluten obtained from different types of wheat or of gluten subjected to various treatments. Usually such measurements are made in order that variations in baking quality of different wheats may be explained, or that some short, rapid method of determining quality of wheat and flour may be established. Since neither of these ends has yet been satisfactorily attained, any method that will yield results that differentiate glutens is of value. Experiments on gluten from wheats of widely varying origin have been carried out in the senior author's laboratory over a period of years, and many differences in the physical properties established (1, 3, 4, 5). In many ways, the methods used in these earlier experiments were rather crude as compared with sedimentation and diffusion methods. It was, therefore, of interest to use these better established methods to check some of the results and conclusions from earlier work.

The results of a comprehensive study of gluten protein using sedimentation and diffusion methods (2) showed that, if our earlier conclusions were correct, definite differences would probably be obtained with gluten from various sources. While the results presented in this paper are largely preliminary in nature, they afford evidence supporting the validity of the earlier conclusions, and indicate some of the ways in which research might be directed when equipment and opportunity are available.

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Material and Methods

Three flours were used as sources of gluten for these experiments. The first two were used in the comprehensive study already referred to (2). The first was a patent flour containing 10% protein milled from Swedish soft wheat, and the second was an experimentally milled flour of 16% protein from Marquis wheat grown at Edmonton in 1939. These flours are typical of two major types used in bread-making, and are known to exhibit marked differences in gluten properties. There were, therefore, definite *a priori* reasons for predicting differences in physical properties. The third flour used was an extreme type that had been studied in some detail in earlier work. It was experimentally milled in 1936 from low-protein Garnet wheat which, even when fresh, produced bread of inferior quality. Considerable deterioration had taken place in the three years following milling and, at the time the experiments reported here were carried out, it was of extremely poor quality. The gluten was coarse, open, and dark, and possessed little tenacity and practically no extensibility.

Details of the methods used have been given in an earlier paper (2). Preparations were all made in sodium salicylate solutions at pH 6.8 because this had been shown to be the most satisfactory dispersing reagent used. The reader is referred to the monograph by Svedberg and Pedersen (6) for details regarding the methods employed and to the earlier paper (2) for a discussion of the application of these methods to the study of gluten.

Results

Properties of Gluten from Three Ether-Extracted Flours

In differentiating gluten fractions, diffusion constants and molecular weights obtained from sedimentation were very useful. It seemed that similar data for the gluten from the three samples of flour under investigation in this study might help to differentiate them. Dispersions to be used in such studies were prepared in the usual manner, using 8% sodium salicylate and maintaining all conditions as uniform as possible for the three samples. Since salicylate dispersions of whole gluten are always cloudy and cannot be used directly for many determinations, these dispersions were clarified by adding 2 cc. of saturated magnesium sulphate to 98 cc. of dispersion. This precipitated the less completely dispersed fractions and left a clear supernatant liquid. These clear dispersions were used in all studies reported in this section. The results are presented in Table I.

As was expected from earlier work (2) the fraction of the whole gluten retained in the clarified dispersion was not the same for the three flours. Less than half of the gluten from the Garnet flour was retained in dispersion, while two-thirds of each of the others was retained. Results in Table I do not, therefore, refer to comparable fractions of the three glutens.

The properties of the glutens from Marquis and soft flour, as measured by the data in Table I, are much alike, although the molecular weights obtained

TABLE I

RESULTS WITH GLUTEN DISPERSIONS FROM ETHER-EXTRACTED FLOURS CLARIFIED AT 2% SATURATION WITH MAGNESIUM SULPHATE

Flour	Protein N in clarified dispersion, % of total N	Conc. of dispersion, %	Weight average molecular weight†	D_{20}	s_{20}	Protein molecularly dispersed, %**
Marquis	67	0.52	129,000	4.34	2.60	49
Soft	66	0.50	119,000*	4.32	2.54	46
Garnet	45	0.53	103,000	4.79	2.45	32

* *Average of two runs that gave 118,000 and 120,000.*

** *As in (2) this is taken as the portion of the gluten protein that formed the main component in the sedimentation diagram.*

† *Calculated from sedimentation equilibrium data.*

from sedimentation equilibrium data are probably significantly different. The diffusion and sedimentation velocity data suggest that there were no differences in the mean particle size, but the s_{20} values apply to only about 70% of the total protein included in the clarified preparations. The differences in the glutens, as measured by these methods, are not sufficiently great, however, to account for the known differences in physical properties.

The gluten from the aged, poor quality Garnet flour, however, yielded quite different results. All of these arise, as would be expected, from the fact that a smaller proportion of the total gluten was included in the dispersion used. The lower molecular weight and higher diffusion constant show that the largest particles had been precipitated. The difference does not lie in the proportion of the dispersed protein that was in molecular form, however, since this varied only from 70 to 73% for the three flours, with Garnet giving a value of 72%. Thus, much less of this gluten was retained in dispersion, but no greater proportion of the dispersed material was in molecular form. It therefore seems reasonable to conclude that the average size of the Garnet particles, whether molecularly dispersed or not, was less than the average size of the particles from either of the other glutens. The extensive study (2) had shown that the greater the proportion of the whole gluten included in a fraction, the higher the molecular weight and the lower the diffusion constant. The differences recorded in Table I can therefore be satisfactorily explained without assuming that there is any fundamental difference in the basic properties of the dispersed protein. The dispersions were all shown to be extremely heterogeneous. This does not appear directly from the data given in Table I, but was apparent from diffusion and sedimentation equilibrium results.

While these investigations failed to yield results that would explain the differences in properties of the glutens from the three flours, they substantiated

in every way the conclusions based on evidence obtained by using much less satisfactory methods.

Preliminary electrophoresis runs were made with the three gluten dispersions used in the experiments reported in Table I. These failed to differentiate the glutens, since the diagrams were similar and the differences in mobility not marked enough to be considered significant.

Effects of Ether Extraction and of Added Linolic Acid as Determined by Sedimentation Measurements

In an earlier paper (2), it was stated that ether extraction of the flour before washing out the gluten had no effect on the solubility or other properties of the gluten. When extraction was first carried out, however, technical grade ether was used, and the solubility of the gluten obtained from the extracted flour was decidedly reduced. This ether contained considerable peroxide, and this was presumably the agent causing the deleterious effect on the gluten. The use of reagent grade ether overcame the difficulty.

These results immediately suggested that the ultracentrifuge might prove useful in checking the validity of conclusions reached in earlier work regarding the importance of lipoidal substances in determining the physical properties of gluten (1, 4). These studies had led to the conclusion that fresh, high-quality gluten owes its properties of coherence, extensibility, and elasticity at least in part to the lipoids that are adsorbed on the gluten to form a protein–lipoid complex. It had also been concluded that these lipoidal substances are susceptible to decomposition as flour ages, and that this decomposition affects the quality of the flour and gluten in two ways. First, as the lipoids decompose they become unavailable for forming the complex; and second, the decomposition gives rise to fatty acids that compete with the remaining undecomposed lipoids for adsorption bonds of the protein. These two factors, although different in their action, both contribute to a gradual degradation of gluten quality, which is shown by a loss in extensibility and coherence, and by the development of a short, open structure.

Gluten, washed from each of the three flours after ether extraction, was dispersed in 8% sodium salicylate. Dispersions were also made from gluten of non-extracted soft and Garnet flours and from the gluten of soft flour after extraction with the technical grade ether. These dispersions were each used in the high-speed ultracentrifuge at 65,000 r.p.m. The results are presented in Table II.

Protein concentration, within the limits used, had already been shown to have little or no effect on the sedimentation constant and no effect on the percentage of the total protein that was molecularly dispersed (2). It is questionable whether any of the differences in sedimentation constants are indicative of real and explicable differences in the gluten. Extraction of the flour appeared to reduce the s_{20} value for soft flour gluten and raise it for Garnet gluten, so no definite conclusion can be drawn. It is probably sufficient

TABLE II

SEDIMENTATION RESULTS WITH GLUTEN FROM THREE FLOURS (8% SODIUM SALICYLATE USED AS SOLVENT)

Run No.	Flour	Treatment	Protein conc., %	$s_{20} \times 10^{13}$	Protein molecularly dispersed, %
12	Soft	Non-extracted	2.00	2.59	68
13	Soft	Non-extracted	1.00	2.60	68
21	Soft	Extracted, technical ether	2.12	2.47	46
22	Soft	Extracted, reagent ether	1.06	2.47	68
36	Garnet	Non-extracted	1.52	2.44	34
35	Garnet	Extracted, reagent ether	1.19	2.53	48
34	Marquis	Extracted, reagent ether	1.14	2.61	62
41	Marquis	Extracted, reagent ether	1.11	2.58	62
53	Marquis	Extracted, + 0.5% linolic acid	1.54	2.55	53
52	Marquis	Extracted, + 1.0% linolic acid	1.58	2.61	49
51	Marquis	Extracted, + 2.0% linolic acid	1.56	2.69	49

to say that there are no major differences in the sedimentation constants for any of these glutens.

The important results are those in the last column of Table II. The marked differences in the percentage of the protein that was molecularly dispersed under similar conditions of centrifuging show that the glutens differed widely in solubility. The molecularly dispersed portion of the gluten from non-extracted Garnet flour was just 50% as large as that of the gluten from either extracted or non-extracted soft flour.

These results show beyond any reasonable doubt that solubility of the gluten depends on more than the protein alone. Four different conditions of the gluten complex can be considered, and all four of these conditions are represented in the results here presented.

First, gluten from normal fresh flours contains such a preponderance of relatively insoluble lipoids (not affected by ether) over fatty acids that the physical properties of the washed gluten are more or less optimum. This condition existed for the gluten used in runs No. 12, 13, 22, 34, and 41. From 62 to 68% of the gluten was molecularly dispersed, the difference between soft and Marquis gluten possibly being related to differences in the natural lipoids or possibly to differences in the proportions of the fractions of the protein itself (3, 4).

Second, by adding linolic acid to a normal flour before washing the gluten, a condition is created in which relatively large amounts of both the natural lipoids and added fatty acids are competing for the adsorption bonds. This is found in runs No. 51 to 53. The solubility of the gluten is reduced, but there appears to be a limit beyond which increased additions of acids are not effective. Even in very badly deteriorated flours fatty acid accumulation

rarely reaches 1% and therefore a 2% addition represents an entirely abnormal condition. It appears, however, that the fatty acids can reduce the solubility of the gluten considerably even when the lipoids necessary for normal gluten formation are present.

Third, by extracting the aged Garnet flour, there is obtained a gluten that is not affected by fatty acids, but in which a considerable portion, if not most of the essential lipoids, has been decomposed during ageing. A similar condition appears to have been brought about by the extraction of soft flour with peroxide-containing ether. The fatty acids are extracted, and it is probable that most of the essential lipoids were decomposed or rendered inactive by the oxidizing action of peroxide. Thus, results for runs No. 21 and 35, although obtained with entirely different glutens, are probably indicative of similar properties of the protein. A marked decrease in percentage gluten dispersed has resulted, as compared with the normal gluten from either soft or Marquis flour.

Finally, the unextracted Garnet flour possesses the most unfavourable properties for obtaining good quality gluten. Most of the essential lipoids have been broken down during ageing, and the fatty acids resulting from the decomposition remain in the flour. The solubility of the gluten obtained under these conditions is very low, and the effects of the two causal conditions appear to be additive. A decrease of about 14% in the molecularly dispersed protein is apparently due to fatty acids, and a further decrease of from 15 to 20% is due to the absence of essential lipoids.

Had the full importance of these results been appreciated at the time the experimental work was performed, or had more time been available, two further types of determinations would have been made. More extensive use of the impure ether would have given more definite evidence of the importance of the substances affected by it; and the addition of fatty acids to flour extracted with such ether and also to the ether-extracted Garnet flour should have established the role of these acids more satisfactorily. It is believed, however, that one may fairly accurately predict the results that would have been obtained.

In earlier studies (4), use had been made of wheat germ to add extra lipoidal substances to gluten. The use of germ had resulted in a marked softening of gluten from high-quality Marquis flour, and in a much improved quality of the gluten from aged, deteriorated flour. The solubility of the latter gluten, as determined by relatively crude methods, was considerably increased. An attempt was made to use germ in the present study, but the germ available failed to give results similar to those obtained earlier. Instead of improving the quality of the gluten from deteriorated flour, it rendered it impossible to handle; and it also reduced the quality and solubility of gluten from Marquis flour. This germ was obtained from soft wheat milled in Sweden, and it seems likely that the properties of such germ are different from those of high-grade Canadian hard wheat. There was insufficient time to secure such germ from Canada, and this work was of necessity discontinued.

Discussion

The results reported in this paper show that, with gluten from different types of flour, sedimentation velocity measurements are of value chiefly because they make possible a determination of the proportion of the protein that is molecularly dispersed. The other methods used in the study failed to distinguish between the glutens to any appreciable degree.

It would appear, therefore, that the properties of the complex that is called "gluten" are determined by much more complicated factors than the basic physical characteristics of the protein itself. Certainly fatty acids and lipoidal substances in general exert a marked effect on the properties of gluten. The role of denaturation in determining the properties of the dispersed gluten was discussed in an earlier paper (2). This factor is not believed to be responsible for the type of result dealt with in the present work. The results obtained in this study confirm earlier conclusions (1, 4) regarding the role of such lipoidal compounds. It seems likely that further studies using sedimentation and diffusion methods would yield valuable information as to the nature of the protein–lipoid complex on which the physical properties of gluten apparently largely depend.

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